

A standard for *Aspergillus* PCR - how to validate the standard

Rosemary A Barnes

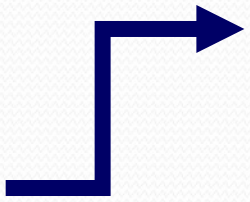
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Diagnostic tests

- Measurement of **analytical validity**
 - the accuracy and precision
 - Sensitivity and specificity
- Evaluation of **clinical validity**
 - the accuracy with which a test identifies or predicts clinical status
 - Diagnostic odds ratio
 - PPV/NPV
- Assessment of **clinical utility**
 - assessment of the risks and benefits
 - cost and patient outcome,
- Consideration of **ethical, legal or social implications**

Royal College of Pathologists. Evaluating and introducing new diagnostic tests. the need for a national strategy. 2006

Diagnostic accuracy

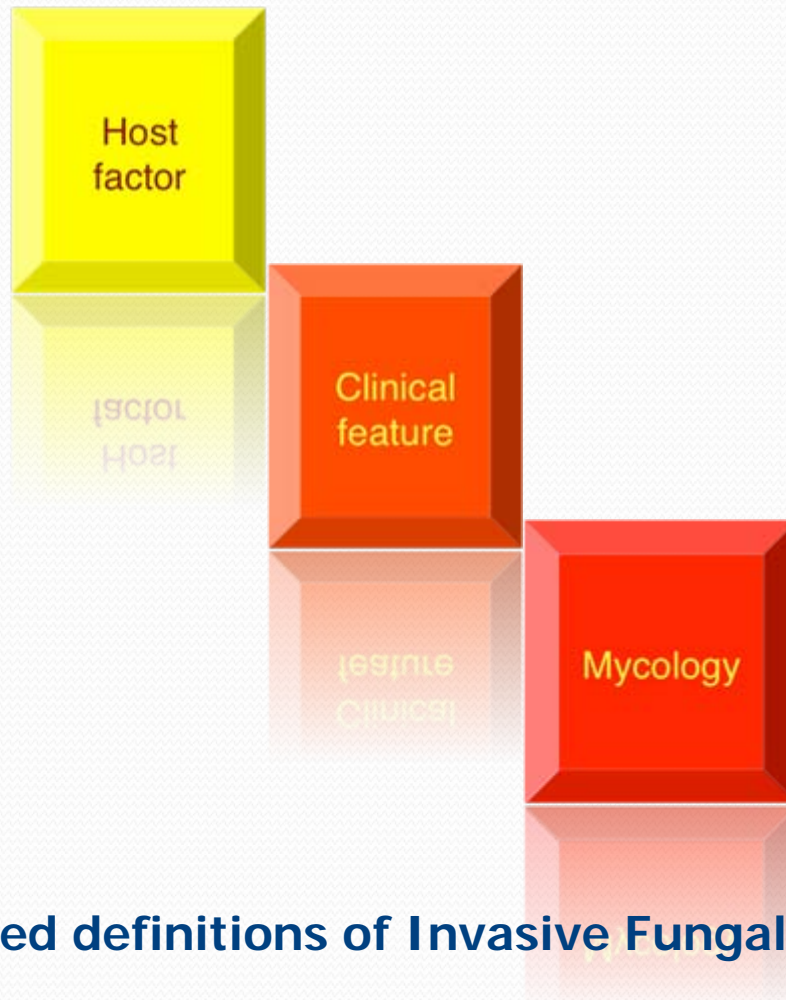
- Sensitivity and specificity
 - quality of the test
 - relatively independent of circumstances
 - predictive accuracies
 - in different practical situations
 - in terms of numbers proportions tested with correct and incorrect results
 - post test probability
- 
- Influenced by
 - Target condition
 - Reference standard
 - Discrimination of test
 - Odds ratio
 - Area under ROC
 - Predictive measures
 - PPV/NPV
 - Likelihood ratio
 - Target population

Diagnostic accuracy

- Benchmarking against reference (**gold**) standard
 - reference standard is available
 - sensitivity and specificity
 - reference standard is available but impractical
 - reference standard is NOT available but consensus standard can be constructed
 - reference standard is NOT available and consensus standard cannot be constructed
 - Calculate measures of agreement

Defining invasive fungal disease

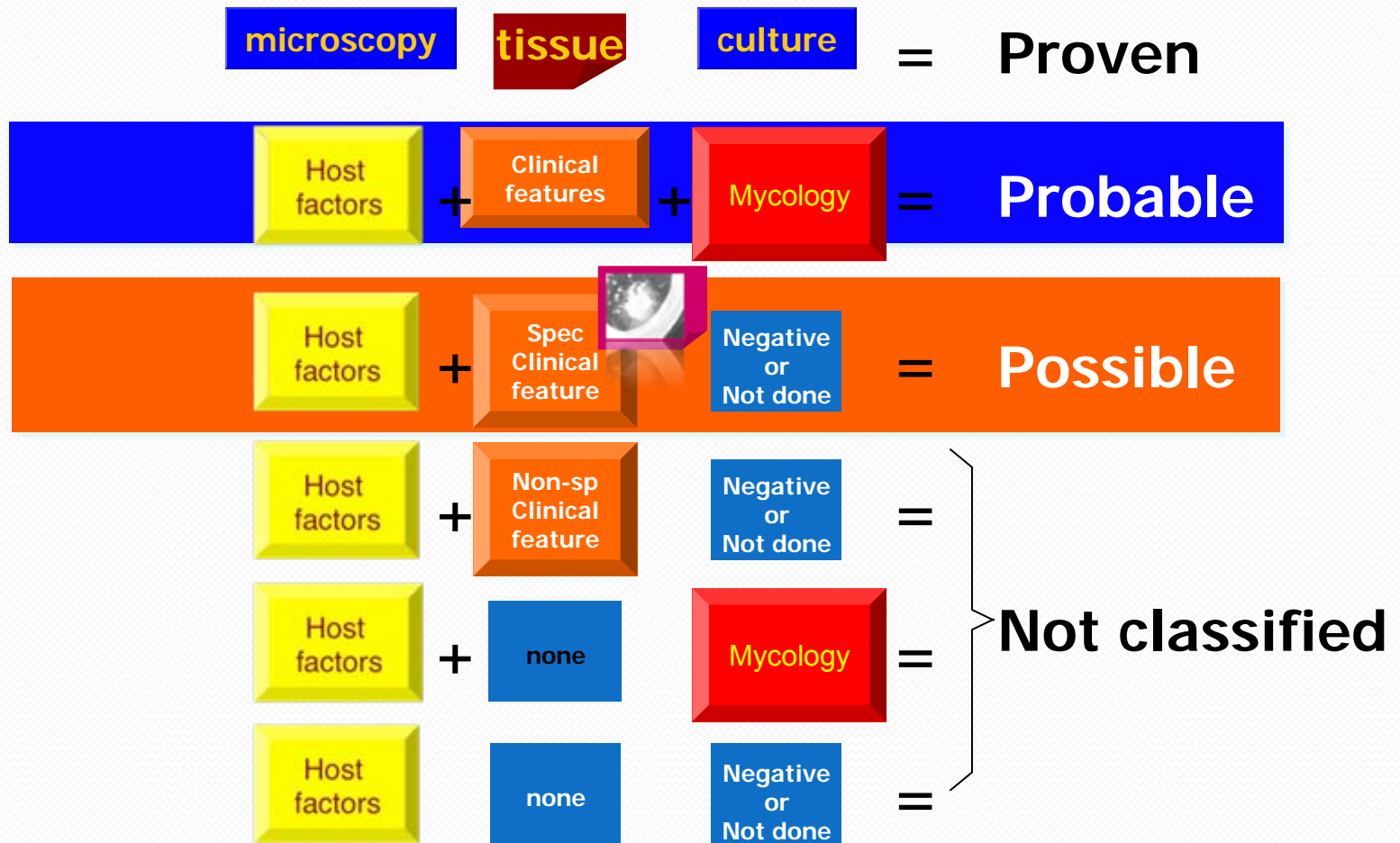
EORTC/MSG Consensus Revised definitions



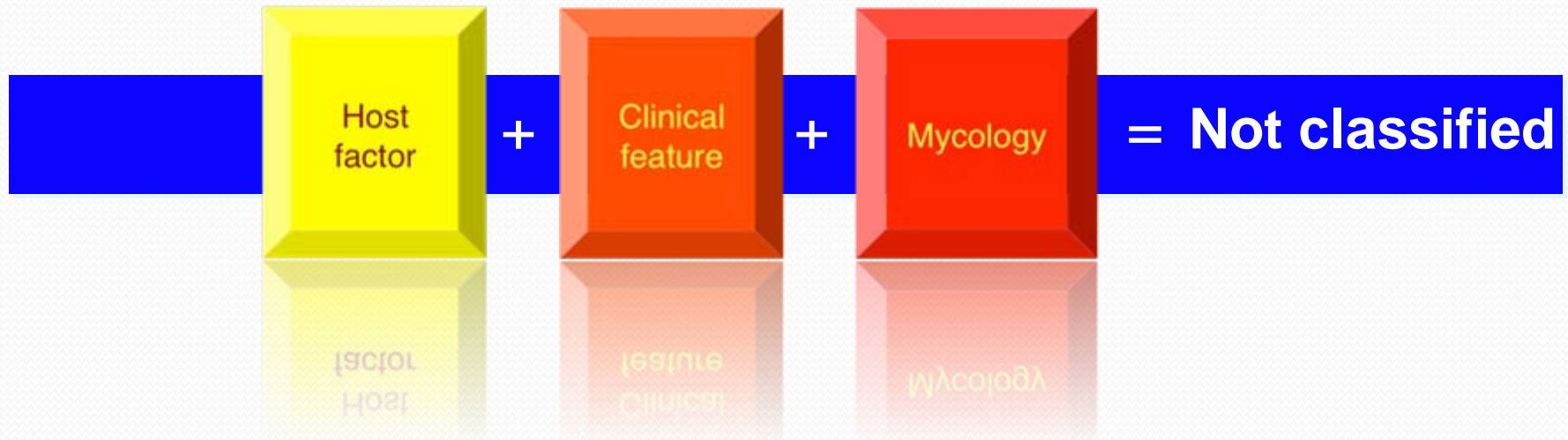
- Aimed to provide definitions for proven , probable and possible fungal infection that could facilitate **clinical research**
 - **Primarily for drug trials**
 - Highly selective population
 - Not representative of real life practice
- Not intended as a **guide to clinical practice**
- Should not be used to reject diagnosis of IFD

Revised definitions of Invasive Fungal Disease de Pauw et al CID 2008:46

Invasive fungal disease - Definitions II

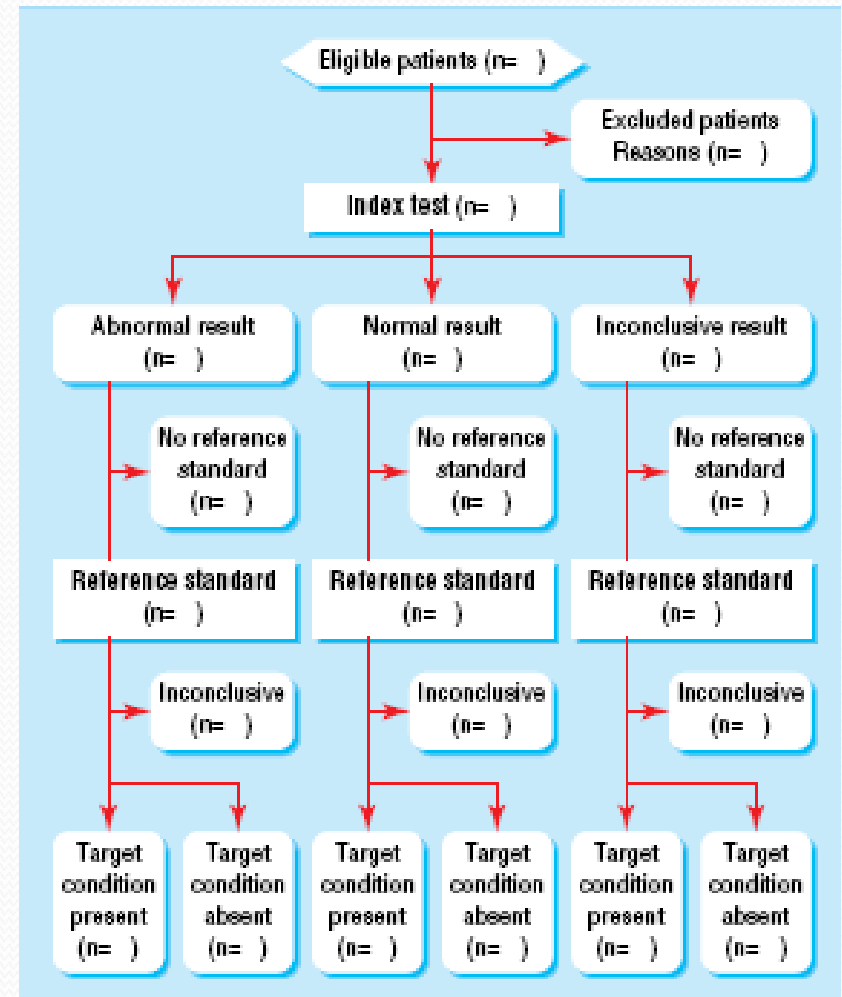


The forgotten ones.....



Standards for Reporting of Diagnostic Accuracy (STARD)

- Designed to improve reporting of studies of diagnostic accuracy
- Provides a checklist of 25 items
 - Title, abstract, and keywords
 - Introduction
 - Methods
 - Participants
 - Test methods
 - Results
 - Estimates
 - Discussion



Factors affecting performance of GM EIA

- OD cut-off used
- HSCT >> SOT
- EORTC/MSG>> non EORTC/MSG
- Paediatric versus adult
- Age of population
- Neutropenia vs non neutropenia
- Prevalence of disease

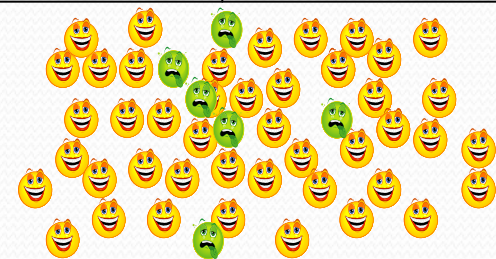
Pfeiffer et al. (2006) Diagnosis of Aspergillosis.
Clin Infect Dis 42:1417-1727

Pfeiffer et al. (2006) Diagnosis of Aspergillosis using GM. Clin Infect Dis 42:1417-1727

	Cases of proven IA		Cases of proven or probable IA	
Prevalence	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
0.05	0.25 (0.23–0.28)	0.98 (0.97–0.99)	0.31 (0.28–0.35)	0.98 (0.97–0.99)
0.10	0.42 (0.39–0.45)	0.96 (0.95–0.97)	0.49 (0.45–0.53)	0.96 (0.95–0.97)
0.15	0.53 (0.50–0.56)	0.95 (0.94–0.96)	0.61 (0.57–0.64)	0.93 (0.92–0.94)
0.20	0.62 (0.59–0.65)	0.92 (0.91–0.94)	0.69 (0.65–0.72)	0.91 (0.89–0.92)



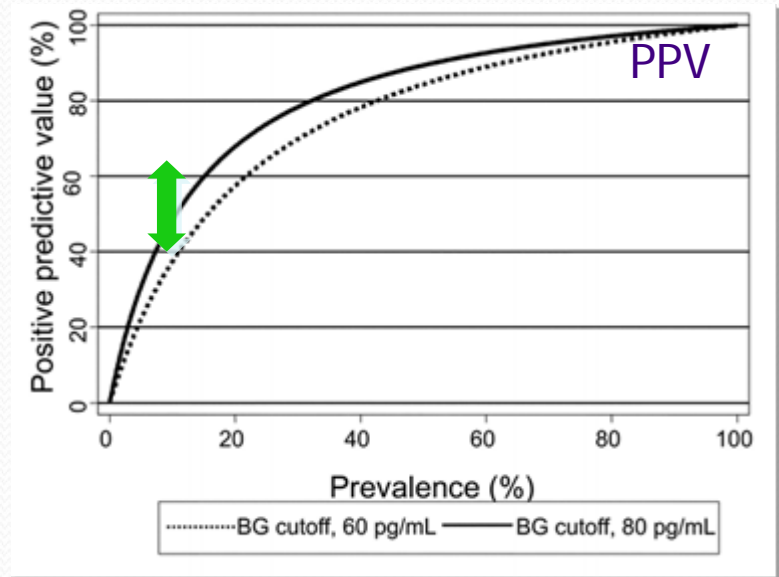
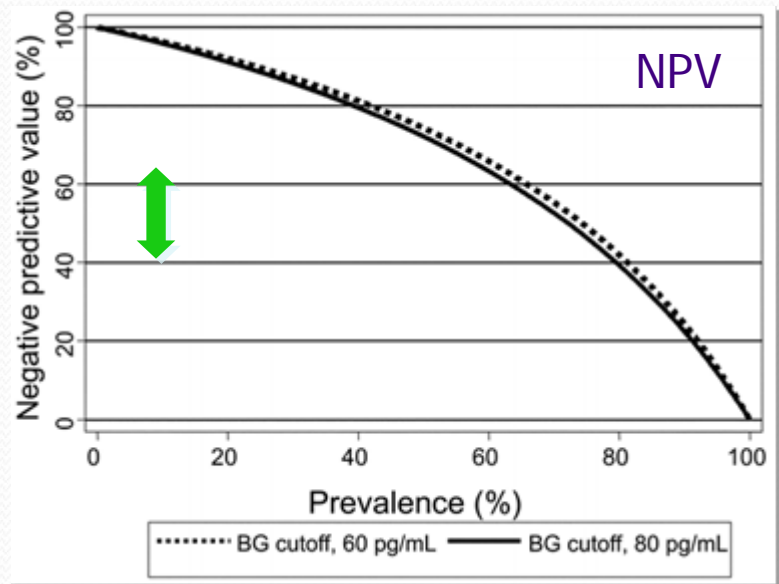
Prevalence: 5%
PPV 25%



Prevalence: 20%
PPV 67%

Prevalence and predictive values

Prevalence 10%



beta-D-glucan test

Study population

- Subjects
 - Risk stratification to identify high risk groups
 - Acute leukaemia
 - Allogeneic SCT
 - (refractory/relapsed lymphoma, aplastic anaemia)
 - Late versus early infection
 - Impact of GvHD, new treatment modalities (CamPath, fludarabine, non-myeloablative transplantation)

Ideal

- Randomized controlled trial
 - Empirical versus pre-emptive therapy strategy
 - According to STARD criteria
 - Investigators blinded to PCR result
 - Antifungal prophylaxis restricted to fluconazole
 - Utilise PCR screening in high risk groups during neutropenia (twice weekly) or in GvHD (biweekly)
 - Independent review committee to apply EORTC/MSG criteria

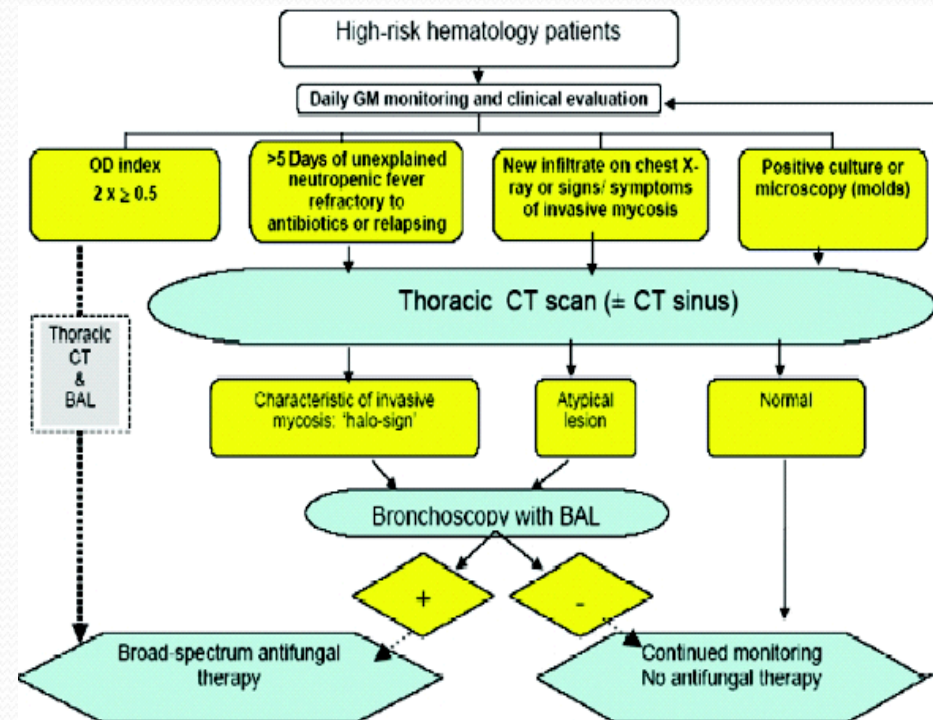
- PCR interpretation
 - Close to a European standard
 - Need to consider how we use these tests
 - Not necessarily to diagnose IFI
 - PPV similarly affected by prevalence of disease but NPV remains high
 - But as a screen to rule out IFI
 - Empirical treatment (and prophylaxis in areas of low prevalence) become unnecessary
- Can biomarkers be used in patient management

Can biomarkers be used for diagnosis

- PCR assays and immunoassays (GM EIA) have been studied
 - Can diagnostic assays be used to limit empiric therapy
 - Is this safe

Galactomannan EIA

- Open study
- 136 episodes of neutropenia
 - Patients receiving flucon prophylaxis
- daily EIA GM + early CT scanning in neutropenic febrile episodes
- Antifungal given if 2 consecutive EIA GM results +ve (index ≥ 0.5)
- and confirmed by BAL or CT



Maertens et al

- 35% of episodes met criteria for empirical antifungal but only 7.7% treated on basis of pre-emptive therapy
- Duration of fever not affected
- 22 cases of IFD only one missed
- 3 breakthrough infections
 - 2 candidaemias
 - 1 mucorales
- No excess mortality or fungal related death
- No impact on overall antifungal usage despite decreased empirical use

Cordonnier *et al* CID 2009 48:1043

- 293 patients randomised
- empirical or pre-emptive therapy
 - empirical arm received antifungals if they had persistent/recurrent fever after 4 days
 - pre-emptive patients given antifungal only if they showed
 - clinical and radiological signs of pneumonia/sinusitis
 - positive GM index ≥ 1.5
 - Aspergillus colonization
 - Septic shock
 - CNS signs/periorbital inflammation
 - Diarrhoea/mucositis \geq grade 3
 - fever > 14 days
- Allo SCT patients were excluded
- Less than 50% received antifungal prophylaxis
 - Mainly non-absorbable
- Most were not neutropenic at baseline

Cordonnier *et al*

- Survival was not significantly different
- “Non inferiority” demonstrated
- pre-emptive patients had more IFD
 - 9.1% vs 2.7%
- pre-emptive patients received significantly less antifungals
- no significant cost savings were achieved
 - Used ampho B deoxycholate first -line

PCR

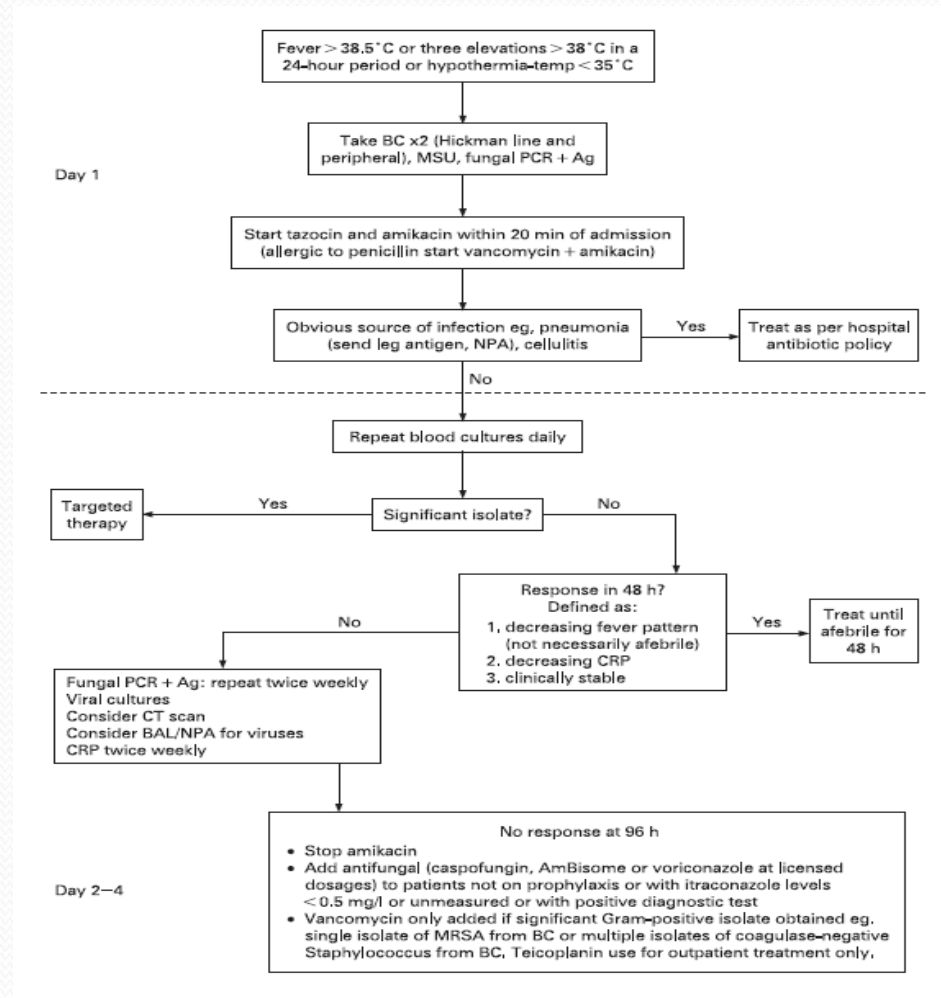
- Allo-SCT randomised to receive lipid Ampho B after either:
 - Single positive PCR or 120 hours of refractory fever (pre-emptive)
 - 120 hours of refractory fever (empirical)

	Antifungal therapy	Proven fungal infection	30 day* mortality
Pre-emptive	57.1%	12	1.5%
Empirical	36.7%	16	6.3%

* No difference at day 100

PCR and Galctomannan

- PCR and EIA incorporated into neutropenic fever care pathway
- Empirical antifungal arm removed for:
 - Patients on effective prophylaxis
 - Itraconazole with serum levels ≥ 0.5 mg/l
 - AmBisome 7mg/kg weekly
 - Unless directed by positive diagnostic test or clinical signs
- One-year follow up



- 125 high risk patients studied prospectively
 - EORTC/MSG criteria
 - 1 year follow up
- Proven/prob IFD 8%
 - 12% if PCR included
- no IFD or excess mortality in patients in whom antifungal treatment was withheld
- Decrease in antifungal use and cost saving

Proven prob disease	PCR	PCR + EIA
Sensitivity (%)		
Single specimen	87.5	100
Multiple specimen	75	87.5
Specificity (%)	98	100
Likelihood ratio		na
Single specimen	3.8	
Multiple specimen	8.3	
DOR		na
Single specimen	21.1	
Multiple specimen	30.7	

Follow up 2005 -2009

- 589 high risk patients screened
 - 95 positive by PCR and ELISA
 - >40% proven/probable aspergillosis
 - 10% positive simultaneously
 - 14% EIA first biomarker positive
 - 76% PCR first biomarker positive
 - Clinical signs are late (except in sinusitis)
 - 308 patients negative by all tests
 - No IA
 - One case of non-aspergillus mould infection (positive by histology and investigational pan fungal PCR test)

Grey (equivocal) areas

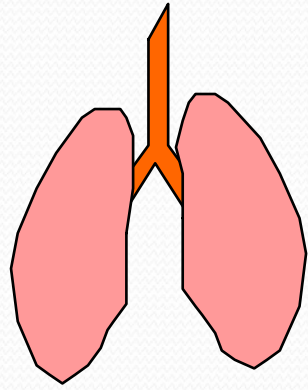
- 59 multiple specimens positive by PCR only
 - 32 (54%) had additional clinical and mycological signs
- 3 multiple specimens positive by EIA only
 - 1 (33%) had additional clinical and mycological signs
- 112 patients with single positive test (PCR or EIA)
- 12 patients unevaluable (only single specimen sent)

Interpretation

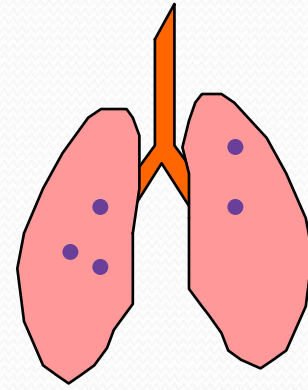
- Designed as a screening test
 - to rule out FI and limit empiric antifungal usage
- Does have some utility as a diagnostic test
 - Provided limitations are understood
- Single positive biomarker
 - indication for a repeat specimen only
- Single specimen positive by both PCR and EIA or multiple specimens positive
 - Antifungals indicated in symptomatic patient
 - Indication for HRCT/BAL if clinical signs absent

Biomarkers to select early treatment

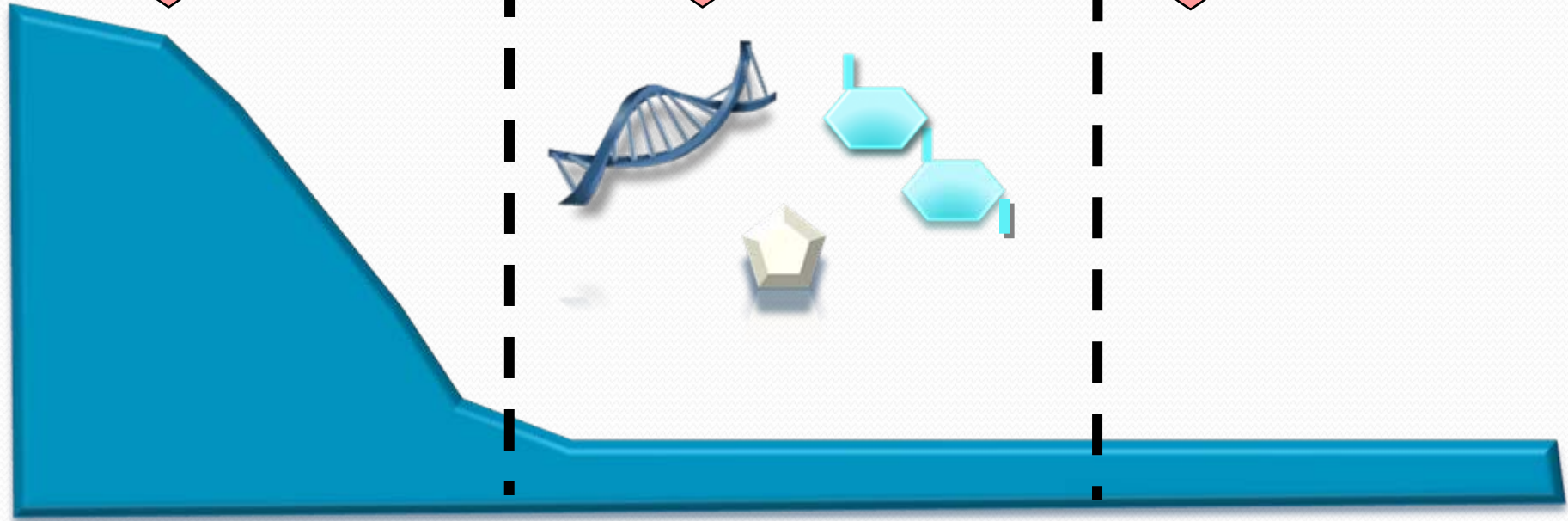
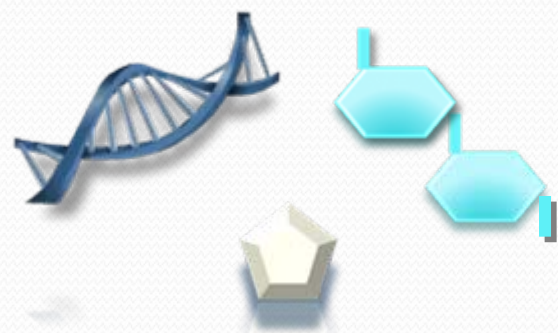
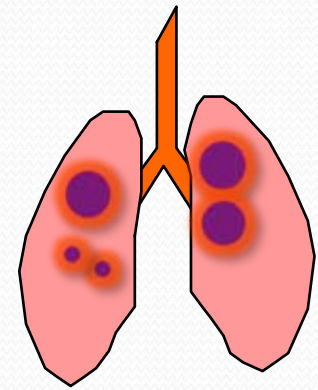
at risk



infection

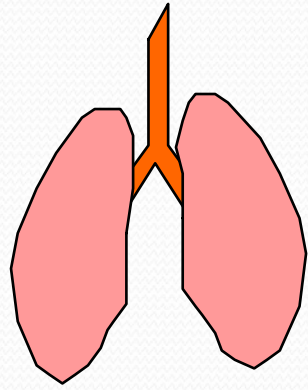


disease

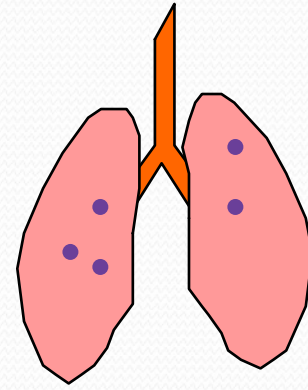


Biomarkers to select early treatment

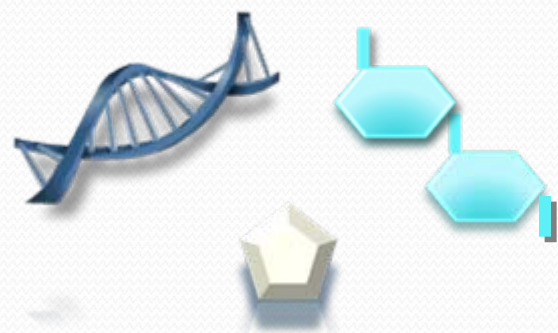
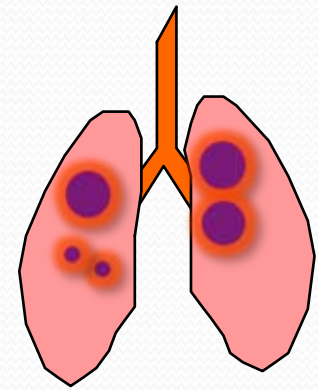
at risk



infection



disease



Prophylaxis

Pre-emptive

Targetted

Prophylaxis

Pre-emptive

Targetted

Potential pitfalls

- using the terms “sensitivity” and “specificity” to compare new test to non-reference standard
- discarding equivocal results when calculating diagnostic accuracy or agreement
- using outcomes that are altered or updated by discrepant resolution to estimate the sensitivity and specificity of a new test
- comparing results of new test to outcome of a testing algorithm that combines several comparative methods if the algorithm uses the new test
- Impact of antifungal drugs (as prophylaxis or treatment)

Useful references

- Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *BMJ* 2003;326:41-44
- **Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests**
2007 <http://www.fda.gov/cdrh/osb/guidance/1620.pdf>.
- **The evaluation of diagnostic laboratory tests and complex biomarkers. Summary of a Diagnostic Summit**
Royal College of pathologists
2008 www.phgfoundation.org

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